AN ELECTRON SPIN RESONANCE STUDY OF COPPER VALENCE IN OXYHEMOCYANIN[1]

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Oxygen combines with hemocyanin in a ratio of one molecule per two atoms of cuprous copper. When the copper is released from oxyhemocyanin by acid denaturing reagents, one of the two copper atoms is found in the cupric state (1). It has therefore been inferred that one of the two copper atoms in the oxygen-combining site of native oxyhemocyanin is in the cupric state. We have now re-examined this matter using electron spin resonance spectroscopy.

Materials. Hemocyanin was obtained from Cancer magister and purified by a method previously described (2). The pure hemocyanin thus obtained was dialyzed overnight in a cold room against M/15 phosphate buffer, pH 6.5, and used immediately, for the experiments. Oxyhemocyanin and deoxyhemocyanin were obtained by passing tank oxygen or catalytically purified hydrogen through a rotating gas exchanger without bubbling. Methemocyanin (3) was prepared by titrating deoxyhemocyanin with H_2O_2; it had a greenish color with an absorption maximum at 625 mμ. Apohemocyanin was prepared by dialysis of deoxyhemocyanin against anaerobic, neutral cyanide (2). Apohemocyanin-copper complex was made by dialyzing 0.1 mM apohemocyanin against 0.1 mM $CuSO_4$ at 5° for 24 hours. It contained 29 atoms of copper per molecule, 16% over the native hemocyanin, (2) but only 21% of its oxygen-binding capacity could be recovered by reduction with ascorbate, which entirely decolorized the anaerobic complex.

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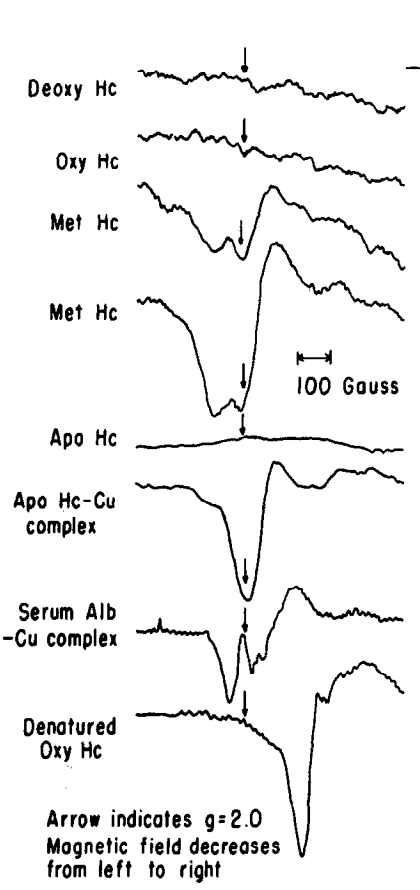

Methods. Electron spin resonance spectroscopy was carried out with a Varian V-4500 EPR spectrometer, using 100 kc. modulation. The sample cell was a quartz cuvette 0.9 x 0.5 x 0.01 inch (7.5×10^{-2} ml) positioned in the cavity to avoid power losses due to water. Field strengths were measured with a Varian F-8 fluxmeter, using nitrosyl disulfonate as a standard. Other pertinent spectrometri details are given in the Figure. All measurements were conducted at room temperature, with samples in M/15 phosphate buffer, pH 6.5 or M/10 acetate, pH 6.0.

Results. Neither deoxyhemocyanin nor oxyhemocyanin gave detectable electron spin resonance spectra, but identical concentrations of oxyhemocyanin denatured in glacial acetic acid gave a strong cupric ion spectrum. Upon titratin deoxyhemocyanin with H_2O_2, a broad signal slowly developed. This was the spectrum of a cupric complex because it had the same g value and characteristics shown by

cupric-apohemocyanin complex, and it was similar to the spectrum shown by serum albumin-cupric complex. Apohemocyanin itself gave no signal.

Conclusions. We have found that when the copper attached to hemocyanin is in the cupric state it is paramagnetic and has an electron spin resonance spectrum. The copper in oxyhemocyanin cannot be in an identical state because it shows no spectrum; acid denaturation clearly produces a change in the electron configuration of this copper.

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